Assessment of the Physicochemical Properties and Antioxidant Content in a Variety of Omani Honey Samples

Haider A. J. Al Lawati*[a], Javad Hassanzadeh[a], Nafiseh Bagheri[a], Muadh Al Shaidi[a], Majid Al Amri[a], Hassan Talib M. Al Lawati[b]

a)Department of Chemistry, College of Science, Sultan Qaboos University, Box 36, Al-Khod 123, Oman

b)Ministry of Agriculture, Fisheries and Water Resource, Box 2446, postal code 112 Ruwi, Oman

Abstract: Honey, a natural product with effective medicinal properties and exceptional energy content, holds a significant place in the hearts of consumers. This study evaluated the pH, free acidity, conductivity, sugar composition, and total antioxidant content of eight types of rare honey produced in Oman. Additionally, well-known types of honey such as Sidr and Sumur, along with some commercial honey samples, were studied for comparison purposes. A simple and innovative paper-based analytical device, recently developed by our group, was applied as an appropriate alternative to time-consuming chromatography-based methods. The findings revealed a low sugar content in a type of honey called "Aitman" (30.4±1.0%), while other rare types of honey showed sugar content within the normal range (45-75%). Additionally, fructose was identified as the primary carbohydrate in almost all samples, followed by glucose. The free acidity of the samples was comparable to Sidr and commercial honey samples but was lower than that of Sumur honey. Interestingly, the total antioxidant content of native rare honey (116.9-325.4 meq gallic acid/Kg) was substantially higher than that of all other analyzed varieties (101.5-196.6 meq gallic acid/Kg). These findings provide valuable insights into enhancing the quality of Omani honey. By guiding the industry to produce premium-quality honey, this research can improve the domestic and international standing of Omani honey. To the best of our knowledge, this is the first report on the evaluation of these rare types of honey produced in Oman.

Keywords: Omani honey; Physicochemical properties; Sumur; Sidr; Rare honey.

تقييم الخصائص الفيزيائية والكيميائية ومحتوى المواد المضادة للأكسدة في مجموعة متنوعة من عينات العسل العماني.

حيدر أحمد جعفر اللواتي*[أ]، جواد حسن زاده [أ]، نفيسة بخاري [أ]، معاذ الشيدي [أ]، ماجد العامري [أ]، حسن طالب اللواتي [ب]

الملخص: العسل، منتج طبيعي ذو خصائص طبية فعالة ومحتوى طاقة استثنائي، يحتل مكانة كبيرة في قلوب المستهلكين. قامت هذه الدراسة بتقييم درجة الحموضة، والحموضة الحرة، والتوصيل الكهربائي، وتركيب السكريات، والمحتوى الكلي للمواد المضادة للأكسدة لثمانية أنواع من العسل النادر المنتج في عمان. بالإضافة إلى ذلك، تم دراسة أنواع معروفة من العسل مثل السدر والسمر، جنبًا إلى جنب مع بعض عينات العسل التجاري لأغراض المقارنة. تم استخدام جهاز تحليلي بسيط ومبتكر يعتمد على الورق، طورته مجموعتنا مؤخرًا، كبديل مناسب للطرق التي تعتمد على الكروماتوغرافيا والتي تستهلك الوقت. كشفت النتائج عن انخفاض محتوى السكر في نوع من العسل يسمى "عيتمن" (30.4±1.0%)، بينما أظهرت أنواع العسل النادرة الأخرى محتوى سكري ضمن النطاق الطبيعي (45-75%). بالإضافة إلى ذلك، لوحظ أن الفركتوز يشكل مصدر الكربوهيدرات الرئيسي في جميع العينات تقريبًا، يليه الجلوكوز، كما كانت الحموضة الحرة للعينات مماثلة لعسل السدر وعينات العسل التجاري لكنها كانت أقل من تلك الموجودة في عسل السمر. ومن المثير للاهتمام أن المحتوى الكلي للمواد المضادة للأكسدة في العسل النادر المحلي (116.9-325.4 مل مكافئ حمض الجاليك/كجم) كان أعلى بكثير من جميع الأنواع الأخرى التي تم تحليلها (101.5-196.6 مل مكافئ حمض الجاليك/كجم). تقدم هذه النتائج رؤى قيمة لتحسين جودة العسل العماني. من خلال توجيه الصناعة لإنتاج عسل عالي الجودة، يمكن لهذا البحث أن يحسن من المكانة المحلية والدولية للعسل العماني. على حد علمنا، هذا هو التقرير الأول عن تقييم هذه الأنواع النادرة من العسل المنتجة في عمان.

الكلمات المفتاحية: العسل العماني، الخصائص الفيزيائية والكيميائية، السمر، السدر، العسل النادر.

1. Introduction

Honey, a natural sweetener produced by bees, is recognized globally for its health benefits due to its well-documented medicinal and nutritional properties. Its antibacterial, anti-inflammatory, and antioxidant properties have made it a preferred choice among health enthusiasts and practitioners alike [1]. This golden elixir is believed to tackle a range of health conditions and is thought to fortify the immune system, nourish the skin, enhance memory, provide natural energy, alleviate sinus problems, and prevent/control Eczema [1]. Honey is deemed a valuable source of nutrition due to its rich content of vitamins, minerals, and enzymes. Honey derived from natural beehives is free of toxins that may be found in factory-produced bottled honey [2].

The composition of honey varies depending on the nectar source(s). It primarily consists of sugars, particularly fructose and glucose, which make up approximately 40-75% of its composition. Additionally, it contains a mixture of amino acids, vitamins, minerals, iron, zinc, and antioxidants. Water is also a significant component of honey, accounting for 15-20% of its composition [3-4].

Honey in Oman is classified based on its source, with the two prominent varieties being Sidr and Sumur, [5]. Each variety is distinguished by its distinct color, thickness, and flavor. Sumur honey is produced during the summer by bees that collect nectar from the *Acacia tortilis* (Forsskal) Hayne tree, the most commonly found wild tree species throughout the country [5-6]. On the other hand, Sidr honey is a highly valued delicacy in the Middle East, primarily sourced from the "Sidr" tree (*Ziziphus spina-christi*), found in various regions of Oman. The distinctive flavour and aroma of this wild honey are attributed to the diverse range of flora and fauna present in its natural environment. Traditionally, the quality of Omani honey has been evaluated through sensory tests such as aroma, taste, color, and texture [7]. Recently, some reports on the physical and chemical parameters of Omani honey have been published, mostly limited to the two types of honey, Sidr and Sumur. For example, Al-Farsi et. al. analyzed 58 honey samples of Sumur and Sidr from various regions in Oman and examined their physicochemical properties. The study revealed that over 64% of the samples had high acidity levels or abnormal total sugar amounts [7]. Similarly, another research article evaluated seven honey samples - four marketed and three locally produced in Oman (Sumur, Sidr, and Zah'r samples) - for their physicochemical properties. Few samples did not meet the required acidity limits and total sugar content [8].

However, the production of honey in Oman is not limited to these two types. There are also rare types of honey sourced from native beekeepers, which are claimed to have high quality and are usually much more expensive than the common Sidr and Sumur varieties. Despite their reputed quality, there has been no study conducted on these rare types of Omani honey.

Our group has previously developed simple and reliable paper-based analytical devices (PADs) for measuring sugars and antioxidants [9-11]. These assays have been tested on honey samples with satisfactory results. Therefore, these methods can be easily applied as an appropriate alternative to time-consuming and expensive chromatography-based techniques for checking the sugar levels and total antioxidant content of honey samples. This study describes the evaluation of important characteristics of eight types of rare Omani honey, listed in Table 1, along with their bee Bee forage plant, locations in Oman, and flowering month. Several commercial, Sidr, and Sumur samples were also evaluated for comparison purposes. The new PAD recently developed by our group [11] was applied to measure the concentrations of different sugars and total antioxidant levels. The pH, acidity, and conductivity of the samples were also measured using common methods. To the best of our knowledge, this is the first report on the evaluation of these rare types of Honey.

Table 1. List of 8 rare types of Omani honey, the Bee forage plant, the location of these plants and the month of flowering [12,13].

No.	Honey name	Bee forage plant (Scientific name)	Place	Season (flowering)
1	Al Zahrat Al Arbaa	A mixture of four plants: a- Zygophyllum simplex b- Calotropis procera c- Ziziphus hajarensis d- Teucrium polium	a-Common after rain in gravel and sandy areas at altitudes up to 300 m b-Common on sandy soils and gravel plains at altitudes up to 500 m c- No. 4 d- No. 7	April/ June Most year April Feb - May
2	Zahrat Al rub'a al Khali	Teucrium polium	Common in desert wadi and rocky mountains altitudes up to 2000 m	Feb - May
3	Zuhoor Rub' al Khali	A mixture of three plants: a-Zygophyllum simplex b- Calotropis procera c- Ziziphus hajarensis	a-Common after rain in gravel and sandy areas at altitudes up to 300 m b-Common on sandy soils and gravel plains altitudes up to 500 m c- No. 4	April/ June Most year April
4	Aitman	Tephrosia nubica	Common in gravel plains and wadis in northern Oman, altitudes up to 400 m	December - May
5	Arabic Gum	Acacia sensgal	Scattered in Governate of Dofar only in wadis, rocky slopes, and near water streams. It can be found scattered in wadi and dry hills, especially in Najd.	March - April
6	Talah	Acacia gerradii	Distribute commonly in the Hajar mountains and wadi in cold and semi-cold areas about 1000 m	April/ June
7	Arabic Luban	Boswellia sacra	Arid mountains area of Dofar within the range of cooling winds altitude up to 1000 m	March-May
8	Qasam	Ziziphus hajarensis	It grows on rocky slopes and wadis western and eastern Hajar range (e.g. Jabal Akhdar)	April

2. Material and methods

2.1. Materials and instruments

In this research, analytical-grade chemicals were used for all experiments without any further purification. All enzymes, including GOx (Glucose oxidase, 100 units mg^{-1}), FDH (D-Fructose dehydrogenase, 400–1200 units mg^{-1}), MT (Maltase, 50 units mg^{-1}), and IVT (Invertase, 300 units mg^{-1}) were purchased from Sigma (USA). The stock solution of each enzyme (5 mg mL^{-1}) was prepared in phosphate buffer ($H_2PO_4^-/HPO_4^{2-}$, pH 7, 0.03 M), and kept at 2 ◦C (stable for at least 2 weeks in this condition). If needed the same buffer was used for dilution purposes. TMB (3,3′,5,5′-tetramethylbenzidine, Sigma) stock solution (5 mM in methanol) was prepared daily.

2.2. Determination of sugars' concentrations and total antioxidant content

It should be mentioned that the analyses of sugars and antioxidants in honey samples were conducted using a simple paper-based analytical device (PAD), according to our recently published work [11] with some modifications. The procedures are briefly described below.

2.2.1. Preparation of paper-based detection assays

Filter paper (Whatman, United Kingdom) was used to print desirable designs using an HP (p1102) LaserJet printer. The paper was placed in an oven (180 ºC) for 15 min, causing the printed ink to diffuse into the paper and create hydrophobic barriers. Each printed PAD involved three separate layers. The first layer, with a circle hydrophilic zone (7 mm in diameter), served as the injection layer, and the second layer was positioned under the injection layer to divide and deliver the injected sample toward the different detection zones on the detection layer. For more detail, the reader is referred to [11].

The detection zones were modified with amino-functionalized Fe metal-organic frameworks composited with CeO_2 nanoparticles (CeO_2@NH_2-MIL-88B(Fe)). Briefly, a mixture of Fe^{3+} and 2-amino terephthalic acid in ethanolic solution (10 μL) and a well-dispersed solution of CeO_2 nanoparticles (0.05 mg mL^{-1} in ethanol) were dropped onto each detection zone and left for 1 h, covered properly to prevent the solvent evaporation, to generate crystals. The washing process was conducted by adding 5 μL DMF followed by ethanol, leaving it for 10 min (The process was repeated three times). Finally, the paper dried at 60 °C for 3 h. In the next step, each detection zone was loaded with a colorimetric peroxidase substrate (TMB, 4 µL of its 5 mM solution in methanol). The paper was then stored for 10 min to allow the solvent to evaporate.

For analyzing sugars, each detection zone was modified with a special enzyme solution (4 µL), depending on the sugar being analyzed. For the measurement of glucose, fructose, sucrose, and maltose, GOx (100 U mL^{-1}), FDH (50 U mL^{-1}), mixed GOX/IVT (80 U mL^{-1}), and mixed GOX/MT (100 U mL−1) were applied, respectively. It is better to mention that no enzyme was embedded on the PADs for measuring the total antioxidant level of samples. The PADs were dried for 10 minutes, assembled, and maintained at 4 °C for consequent analysis.

2.2.2. Application of honey samples to PAD

Honey samples (1 g) were dissolved in about 150 mL of deionized water in a 250 ml beaker, which was then filled to the mark with deionized water. The prepared solutions were applied to measure the sugars and total antioxidant levels. The process started with dropping a small volume (60 µL) of the prepared sample solution onto the injection zone. After 3 minutes, the device was turned back, and the generated blue color in the detection zones was recorded using a smartphone. For the determination of the total antioxidant level, 3 µL of H_2O_2 (0.05 M) fresh solution was dropped on the corresponding detection zone and left for another 3 min. A Huawei smartphone (P20 Pro, China) was used to record the generated colors, which were processed by ImageJ software to obtain the average color intensity for each zone. The results were shown as relative light units (RLU), which were then connected to the considered analyte concentration. To ensure reproducible results, the photos were all taken with one smartphone under fixed conditions.

2.3. Acidity and pH

To determine the pH and acidity, 1 g of each honey sample was completely dissolved in 60-70 ml of deionized water in a 100 ml beaker, which was then filled to the mark with deionized water. The solution was used to measure pH using a pH meter (RL060P, Hanna Instruments), and then it was titrated with NaOH solution (0.1 N) up to pH 8.3, using phenolphthalein as the indicator. The acidity of samples was stated as the content of all free acids in meq/kg.

2.4. Conductivity measurement

A solution for each honey sample was prepared as mentioned in the previous section and used to measure its conductivity using a conductometer (HI 9811, Hanna instruments). The conductivity of samples was stated in mS.

2.5. Real samples

Thirty-eight Omani local honey samples including eight rare native kinds (namely “Al Zahrat Al Arbaa”, “Zahrat Al rub’a al Khali”, “Zuhoor Rub' al Khali”, “Aitman”, “Arabic Gum”, “Talah”, “Arabian Luban”, and “Qasam”), eighteen Sumur (Showed as Sm1-Sm18), and twelve Sidr (Showed as Sd1-Sd12), as well as six commercial samples (Showed as C1-C6) purchased from a local supermarket in Muscat, were analyzed. Local honey samples were directly collected from beekeepers from different regions in Oman and stored in a dark dry space at a temperature not exceeding 20 °C. The samples were directly analyzed after proper dilution, as described in each section.

3. Results and Discussion

The pH, free acidity, and conductivity of all samples are tabulated in Table 2. Total antioxidant contents (TAC, meq Gallic acid/Kg) of the examined samples are reported in Table 3 while the percentage of four sugars (Glucose, Fructose, Sucrose, and Maltose) are stated in Table 4 along with the ratio of fructose to glucose percentage.

3.1. pH

The pH of honey is a crucial parameter to study due to its significant effect on its shelf life. Honey naturally has a moderately acidic pH because of the presence of natural organic acids. An acidic pH can also minimize microbial content because of their inability to survive in acidic conditions.

All eight rare kinds of honey showed an acidic pH in the range of 3.61-6.49. The lowest pH values were obtained for Zahrat Al rub’a al Khali (3.61±0.03), and Arabic Gum (3.83±0.04), respectively, while the pH of Al Zahrat Al Arbaa, Qasam, and Aitman was higher than 5 (Table 2). In comparison, all 18 Sumur honey samples analyzed in this research showed high acidic pH values in the range of 3.57-4.45, and most Sidr samples were less acidic (3.56-7.23); four samples had a pH between 5-6, and five samples showed a near-neutral pH (6.46-7.23). Finally, all 6 commercial samples were acidic with pH values of 3.81-4.40 (Table 2). These results are consistent with the previous studies [7-8, 14], which reported that Sidr honey generally has a higher pH (>5) than Sumur honey (<5). Since freshly prepared honey samples were used for the current study, the variation in pH values is not related to the storage conditions but it is likely due to the differences in bee species and their source [15].

The acidity of honey is known to be a crucial factor in its ability to inhibit pathogenic microorganisms, and this is especially true for these Omani honey types. According to the results, the different types of honey found in Oman exhibit varying degrees of effectiveness against bacterial infections. Sumur and most of the native honey samples (Zahrat Al rub'a al Khali, Zuhoor Rub' al Khali, Arabic Gum, Talah, Arabian Luban) showed particularly strong antibacterial properties, possibly due to their acidic pH values (3.5>pH>5.5).

3.2. Free acidity

Honey's acidity is caused by organic acids, especially gluconic acid, which results from the enzymatic oxidation of glucose, as well as inorganic ions such as chloride and phosphate (Al-Farsi et al., 2018). Differences in various honey samples can be attributed to the different acids in various floral varieties. A low free acidity value may be linked to a low rate of undesirable fermentation by osmotolerant yeast to convert glucose and fructose to carbon dioxide and ethyl alcohol, which subsequently decreases the formation rate of acetic acid by atmospheric oxygen. In contrast, a high level of free acidity in honey indicates the existence of internal esters, lactone, and ions such as phosphate, sulfate, and chloride [16]. In other words, the amount of acetic acid formation in honey is indirectly estimated through the measurement of free acidity.

The primary source of mineral ions in honey is the nectar collected by bees from flowers. The composition of nectar includes various minerals and ions, including chloride and phosphate, which are then incorporated into the honey. Bees also contribute to the mineral content of honey through their metabolic processes, by adding enzymes and other compounds. Finally, the soil and water in the region can affect the mineral content of the nectar and honey. Regions with higher levels of ions in the soil or water will likely produce honey with higher concentrations of mineral species. These ions can contribute to the overall acidity and conductivity of honey, affecting its taste, preservation properties, and health benefits [17].

The acidity of Omani rare honey samples ranged broadly from 7.87 to 86.67 meq(GA)/kg; Aitman (7.87±0.23 meq(GA)/kg), and Al Zahrat Al Arbaa (18.20±1.02 meq(GA)/kg) showed relatively low acidity values, while Talah (86.67±3.58 meq(GA)/kg) had an acidity value higher than 50 meq(GA)/kg. The acidity of Zahrat Al rub'a al Khali, Zuhoor Rub' al Khali, Arabic Gum, Arabian Luban, and Qasam were also 32.00±2.14, 42.00±2.76, 40.00±0.96, 23.47±2.11, and 21.47±1.28 meq(GA)/kg, respectively. The acidity of the other analyzed samples also showed a wide range from 5.98-166.67 meq(GA)/kg (Table 2). The highest acidity was obtained for Sumur honey samples, ranging from 67.33 to 166.67 meq(GA)/kg, with twelve samples having acidity values higher than 100 meq(GA)/kg. In contrast, Sidr samples showed very low acidity in the range of 5.98-25.53 meq(GA)/kg, with seven samples having acidity values lower than 10 meq(GA)/kg. Commercial samples also showed relatively low acidity values ranging from 11.00 to 41.00 meq(GA)/kg.

As stated by the Codex Alimentarius [18], European regulations [19], and the Gulf Standardization Organization (GSO) [20], the free acidity of honey must not exceed 50 meq/kg. All 18 Sumur samples and one native rare sample (Talah) exceeded this limit. This high acidity level can also be attributed to the production of organic acids from sugar fermentation, which causes their sour taste. Some groups have reported similar results. For example, Raweh et. al. [21] reported the high acidity of Talah honey produced in Saudi Arabia and correlated it to the floral origin of this honey. Accordingly, it is recommended that the specification of the free acidity limits be reviewed for the Sumur and Talah honey types.

3.3. Conductivity

As one of the primary specifications used to assess the quality of honey, conductivity is useful for distinguishing the purity of honey and its floral origin [16, 22]. Honey contains components such as organic acids and minerals, which in an aqueous solution have the ability to dissociate into ions or to conduct electric power. Thus, the conductivity of honey can be directly correlated with the concentrations of mineral salts, organic acids, and protein, which is beneficial for the classification of honey with various floral origins [23,24]. The most common acids in honey are organic acids, such as tartaric, citric, oxalic, and acetic acids, which influence its acidity and conductivity. Many of these acids are present in the nectar collected by bees, while others, like gluconic acid, result from the enzymatic breakdown of sugars. The predominant acid in honey is gluconic acid, derived from glucose oxidase provided by bees during ripening [25].

Citric acid is also present, and the concentrations of gluconic and citric acids help differentiate floral honey from honeydew [25]. Additionally, levulinic and formic acids are produced from 5-HMF through successive reactions, increasing the free acidity of honey [25, 26].

The data obtained in this research showed that most honey types harvested in Oman have higher conductivity (Table 2), which implies higher mineral content in these samples. The conductivity value of rare Omani samples ranged from 0.82 to 1.73 mS, with one sample, namely "Talah", showing a conductivity value of 2.56±0.03 mS, corresponding to its relatively high level of free acid. Sumur honey also showed relatively high conductivity values, ranging from 1.34 to 2.53 mS, showing a high quantity of organic acids, mineral salts, and proteins compared to other varieties. This result is compatible with their high free acidity values. Thirteen Sumur samples showed a conductivity higher than 2.00 mS, while only five had conductivity values of less than 2.00 mS. Most Sidr samples had conductivity values between 1.00 and 2.00 mS, except two samples (Sd12 and Sd8) which showed conductivity values of 0.45±0.04 and 2.17±0.05 mS, respectively. Commercial samples mostly had conductivity values of less than 0.8 mS, with only two samples showing conductivity values of 1.12±0.06 and 1.13±0.03 mS.

A high conductivity value indicates a high level of organic acids, often apparent from the honey's color. It has been shown that a darker color can be correlated with higher conductivity [26]. Despite the minor amounts of organic acids in honey, they have a significant effect on the honey's physicochemical properties and vary according to the floral origin [16, 27]. Storage time and harvesting conditions can also change the conductivity of the honey.

Table 2. pH, acidity, and conductivity of the examined samples.

Sample code	Sample type	pH		Conductivity (mS)		Acidity (meq/kg)	
		Average	SD	Average	SD	Average	SD
Sm1	Sumur	4.17	0.03	2.40	0.02	99.67	3.79
Sm2	Sumur	4.26	0.04	2.19	0.07	117.33	3.82
Sm3	Sumur	4.42	0.07	2.09	0.02	118.67	6.35
Sm4	Sumur	4.37	0.03	2.33	0.04	96.50	2.18
Sm5	Sumur	3.98	0.03	2.53	0.11	128.83	1.61
Sm6	Sumur	4.03	0.01	2.30	0.10	137.33	6.03
Sm7	Sumur	3.57	0.08	1.83	0.08	166.67	14.43
Sm8	Sumur	4.45	0.04	2.16	0.04	99.00	1.73
Sm9	Sumur	4.01	0.08	2.28	0.14	141.67	7.64
Sm10	Sumur	3.94	0.02	2.29	0.09	141.67	2.52
Sm11	Sumur	4.10	0.07	1.34	0.01	67.33	0.58
Sm12	Sumur	4.21	0.01	1.85	0.04	99.00	5.29
Sm13	Sumur	4.20	0.02	2.02	0.04	108.67	7.57
Sm14	Sumur	4.08	0.00	1.56	0.10	88.00	3.46
Sm15	Sumur	4.07	0.01	2.28	0.18	136.50	1.32
Sm16	Sumur	4.06	0.02	2.18	0.13	132.67	4.73
Sm17	Sumur	4.09	0.03	2.18	0.08	137.00	2.00
Sm18	Sumur	3.89	0.02	1.73	0.22	101.67	2.08
Sd1	Sidr	6.93	0.07	1.35	0.09	6.37	0.87
Sd2	Sidr	6.98	0.28	1.46	0.03	5.98	0.48
Sd3	Sidr	7.23	0.24	1.25	0.03	7.43	0.31
Sd4	Sidr	5.48	0.30	1.12	0.03	9.90	0.66
Sd5	Sidr	5.04	0.04	1.61	0.05	10.70	0.75
Sd6	Sidr	5.97	0.05	1.40	0.04	8.43	0.40
Sd7	Sidr	5.07	0.34	1.31	0.07	9.90	0.17
Sd8	Sidr	4.24	0.05	2.17	0.05	13.27	1.01
Sd9	Sidr	6.68	0.21	1.52	0.03	11.00	0.90
Sd10	Sidr	6.46	0.11	1.63	0.13	7.57	0.31
Sd11	Sidr	4.40	0.01	1.35	0.08	18.47	1.34
Sd12	Sidr	3.56	0.05	0.45	0.04	25.33	0.58

Table 2. Continued.

Sample code	Sample type	pH		Conductivity (mS)		Acidity (meq/kg)	
		Average	SD	Average	SD	Average	SD
C1	Commercial	4.31	0.02	0.31	0.01	16.33	0.58
C2	Commercial	4.40	0.10	0.66	0.04	29.67	1.15
C3	Commercial	4.06	0.15	1.13	0.03	40.00	1.00
C4	Commercial	3.81	0.11	1.12	0.06	41.00	2.09
C5	Commercial	4.00	0.04	0.66	0.03	29.67	1.15
C6	Commercial	3.89	0.01	0.21	0.01	11.00	1.99
R1	Al Zahrat Al Arbaa	5.85	0.07	1.12	0.02	18.20	1.02
R2	Zahrat Al rub’a al Khali	3.61	0.03	1.73	0.03	32.00	2.14
R3	Zuhoor Rub' al Khali	4.07	0.05	1.82	0.01	42.00	2.76
R4	Aitman	6.49	0.03	0.56	0.04	7.87	0.23
R5	Arabic Gum	3.83	0.04	1.63	0.02	40.00	0.96
R6	Talah	5.05	0.13	2.56	0.03	86.67	3.58
R7	Arabic Luban	4.15	0.08	1.02	0.08	23.47	2.11
R8	Qasam	5.98	0.11	0.94	0.05	21.47	1.28

3.4. Total antioxidant content (TAC)

Honey’s antioxidant content can vary depending on the plant type that bees collect nectar from. Thus, environmental conditions such as climate, humidity, and excessive solar exposure can affect TAC [28]. For instance, highly sun-exposed plants typically have significantly high TAC [29]. The antioxidant activity of honey is mostly due to its phenolic compounds, which can scavenge free radicals and protect cells from their detrimental effects. In this research, the TAC of honey samples was evaluated using a simple and fast PAD based on the inhibiting effects of antioxidant compounds on the colorimetric reaction of TMB-H_2O_2 in the presence of CeO_2@NH_2-MIL-88B(Fe), as the catalyst. The higher the TAC, the lower the intensity of color on the paper. This device offers an instrument-free and user-friendly assay to screen the quality of honey.

Sumur and Sidr honey samples did not show a significant difference in their TAC (Table 3). They mostly exhibited TAC values between 100 and 200 meqGA/kg, with average TAC values of 147.13 ± 4.17 meqGA/kg for Sumur honey and 135.96 ± 6.08 meqGA/kg for Sidr honey (Figure 1). The commercial honey samples also showed a similar range of TAC (106.48-193.73 meqGA/kg), with an average value of 160.26 ± 3.62 meqGA/kg. However, significantly higher TAC values were observed for rare varieties (Table 3, Figure 1), with an average amount of 261.74±9.38 meqGA/kg. The highest TAC values corresponded to "Al Zahrat Al Arbaa" (325.41±7.16 meqGA/kg), and "Qasam" (300.68 ± 2.93 meqGA/kg) honey samples, followed by "Talah" (286.41 ± 4.01 meqGA/kg) and "Arabian Luban" (275.06 ± 4.23 meqGA/kg).

These findings demonstrated that certain rare types of honey exhibit notably high levels of antioxidant activity, setting them apart from other commonly available varieties. This suggests that these particular types of honey could be highly beneficial for large-scale production, thus improving the overall quality of Omani honey and enhancing its position both nationally and internationally. This research has important implications for the honey industry, shedding light on the potential health benefits of using native honey varieties in production.

It should be mentioned that phenolic compounds can also influence the acidity and conductivity of honey. However, their relatively low concentrations and weak acidity make their influence negligible.

Table 3. Total antioxidant contents (TAC, meq Gallic acid/Kg) of the examined samples.

Sample code	Sample type	TAC		Sample code	Sample type	TAC	
		Average	SD			Average	SD
Sm1	Sumur	139.35	4.93	Sd1	Sidr	106.83	5.01
Sm2	Sumur	125.68	4.82	Sd2	Sidr	98.43	4.09
Sm3	Sumur	143.42	6.34	Sd3	Sidr	126.89	7.26
Sm4	Sumur	159.37	6.67	Sd4	Sidr	116.76	4.72
Sm5	Sumur	196.58	7.98	Sd5	Sidr	135.86	2.97
Sm6	Sumur	114.08	3.75	Sd6	Sidr	99.92	2.55
Sm7	Sumur	180.86	8.42	Sd7	Sidr	136.54	5.49
Sm8	Sumur	127.26	4.17	Sd8	Sidr	158.43	6.83
Sm9	Sumur	146.53	5.92	Sd9	Sidr	164.2	8.08
Sm10	Sumur	165.46	7.03	Sd10	Sidr	138.49	6.97
Sm11	Sumur	174.69	8.19	Sd11	Sidr	159.43	6.04
Sm12	Sumur	114.16	4.67	Sd12	Sidr	189.74	9.43
Sm13	Sumur	125.69	4.37				
Sm14	Sumur	142.27	5.11	R1	Al Zahrat Al Arbaa	325.41	7.16
Sm15	Sumur	166.49	5.94	R2	Zahrat Al rub'a al Khali	116.87	2.68
Sm16	Sumur	101.52	3.86	R3	Zuhoor Rub' al Khali	254.16	5.41
Sm17	Sumur	138.64	6.34	R4	Aitman	153.71	3.98
Sm18	Sumur	186.35	9.46	R5	Arabic Gum	260.89	3.25
				R6	Talah	286.41	4.01
C1	Commercial	139.07	5.87	R7	Arabic Luban	275.06	4.23
C2	Commercial	193.18	10.31	R8	Qasam	300.68	2.93
C3	Commercial	142.84	6.91				
C4	Commercial	186.28	5.48				
C5	Commercial	106.48	3.94				
C6	Commercial	193.72	6.87				

3.5. Sugar content

The sugar content and profile of honey vary due to different parameters, mainly geographical origin, climate, botanic origin, and processing and storage conditions [4, 24]. Monosaccharides are the most common carbohydrates found in honey, with concentrations of up to 75%. Disaccharides and other sugars can also be present in low amounts (commonly less than 10%). The content and types of sugars in honey can define its primary properties such as viscosity, hygroscopicity, crystallization rate, and energy value [30]. Generally, fructose is the major carbohydrate found in almost all types of honey, except for some varieties that have a higher concentration of glucose, leading to a relatively high crystallization rate. Therefore, fructose and glucose fractions and the ratio between them are commonly used to classify monofloral honey [31].

In this research, the total sugar content and sugar profile of honey samples were simultaneously assessed by the simple and fast PAD, developed in our previous work [11]. Depending on the sugar being analyzed, special enzyme(s) were used to react with sugar and generate H_2O_2, which was then measured based on its oxidizing effect using the colorimetric reaction of TMB-H_2O_2 in the presence of CeO_2@NH_2-MIL-88B(Fe), as the catalyst. The color intensity on the paper increases with higher sugar concentration, allowing for a fast, instrument-free, and user-friendly assay for real-time screening of honey quality. For example, Figure 2 illustrates the color variations corresponding to different concentrations of glucose. It is evident that there is a direct relationship between the intensity of the colors observed on the paper and the respective concentrations of glucose. As the concentration of glucose increases, the intensity of the color exhibits a linear increase when plotted against the logarithm of these concentrations.

The major sugars found in Omani honey are fructose (21.47%-36.93%) and glucose (19.65%-33.52%), along with small percentages of sucrose (6%>) and maltose (5%>). The total sugar content in all examined samples ranged from 48.58% to 72.56%, consistent with the standards of the Codex Alimentarius Committee on Sugars (2001) (Alimentarius, 2001). The highest total sugar contents were observed in samples Sd3 (72.56 ± 2.37%), Sd4 (70.4.9 ± 3.10%), and Sd5 (69.82 ± 3.29%), all belong to the Sidr variety. The sugar profiles for different samples are indicated in Table 4.

The fructose and glucose percentages of Sumur and Sidr samples ranged from 21.47 to 36.93% and from 19.65% to 33.52%, respectively, with average values of 30.41 ± 3.47% for fructose and

26.89±4.08% for glucose. There was no significant difference between the sugar profiles of these two varieties (Figure 3). Rare honey samples also showed similar ranges (22.16-30.01% and 22.40-31.12% (Figure 3), respectively for fructose and glucose), except for the "Aitman" sample, which had relatively low fructose (12.33 ± 1.08%) and glucose (15.07 ± 1.42%) contents compared to their average values of 26.89 ± 2.58%, and 25.46 ± 3.60%, respectively.

According to the standards of the Codex Alimentarius Committee on Sugars (2001), the minimum amount of reducing sugars is 45 g per 100 g for different honey types [18]. Therefore, the unique composition of Aitman honey suggests that it may not be classified as traditional honey but rather a natural product that mimics honey, suitable for individuals seeking to reduce sugar intake. Besides, the sucrose and maltose contents of rare samples were less than 4.5% and 3.5%, respectively. For commercial samples, fructose and glucose contents ranged from 26.35% to 33.47% and from 24.72% to 30.06%, respectively, with sucrose and maltose contents less than 6% and 3.5%, respectively. However, one sample had a sucrose concentration of 8.63 ± 0.57%, which was higher than the others.

The obtained results confirmed that the sugar contents of all the Omani honey samples are within normal ranges, with the sums of fructose and glucose contents falling within the 45-75% range [18]. The presence of a high percentage of sucrose in honey is possibly attributed to bee artificial feeding [4], however, it was reasonably low in the examined Omani samples. Additionally, in most cases the fructose content exceeds the glucose amount, showing good apparent quality. However, in some cases (7 of 44 examined samples, including Sm3, Sm4, Sd2, Zuhoor Rub' al Khali, Zahrat Al rub'a al Khali, Aitman, and one commercial sample), showed a fructose-to-glucose ratio lower than 1, indicating relatively fast crystallization, thus dropping the honey quality. This is because of less solubility of glucose in water than fructose.

One native honey sample (Aitman) showed different results; it has a pH of 6.49, a total sugar content (Glucose + Fructose) of 27.41%, and a total antioxidant activity of 153.71 (meq GA/Kg). Considering the less acidic environment, lower sugar content, and moderate antioxidant activity, this type of honey is expected to have relatively lower antibacterial properties compared to other examined honey samples. In contrast, it can be useful for individuals seeking to reduce sugar intake.

Table 4. The sugar profiles of the examined samples.

Sample code	Sample type	Glucose (%)		Fructose (%)		Sucrose (%)		Maltose (%)		F/G
		Average	SD	Average	SD	Average	SD	Average	SD	
Sm1	Sumur	24.62	0.78	28.71	0.91	<2	-	1.95	0.07	1.17
Sm2	Sumur	25.65	0.64	27.11	0.71	3.16	0.23	<1	-	1.06
Sm3	Sumur	27.24	0.84	28.81	0.57	2.98	0.15	2.26	0.09	1.06
Sm4	Sumur	23.16	0.74	21.47	0.85	3.95	0.20	<1	-	0.93
Sm5	Sumur	28.86	0.69	32.62	0.93	2.44	0.16	2.39	0.05	1.13
Sm6	Sumur	27.41	0.82	31.97	1.14	<2	-	<1	-	1.17
Sm7	Sumur	30.15	1.27	32.66	0.84	4.16	0.18	2.41	0.08	1.08
Sm8	Sumur	23.46	1.06	29.44	0.92	<2	-	1.93	0.09	1.25
Sm9	Sumur	27.76	0.98	29.95	1.08	3.57	0.15	2.36	0.14	1.08
Sm10	Sumur	28.62	1.03	34.51	1.51	3.92	0.22	1.46	0.11	1.21
Sm11	Sumur	25.38	0.84	26.64	0.93	4.44	0.09	<1	-	1.05
Sm12	Sumur	21.87	0.92	21.74	0.71	6.62	0.24	3.51	0.20	0.99
Sm13	Sumur	26.71	0.88	30.04	1.05	2.95	0.19	<1	-	1.12
Sm14	Sumur	24.93	1.05	28.74	0.95	<2	-	2.71	0.12	1.15
Sm15	Sumur	28.62	8.63	34.12	1.47	4.61	0.13	<1	-	1.19
Sm16	Sumur	21.36	6.08	27.54	1.21	<2	-	3.38	0.22	1.29
Sm17	Sumur	22.67	1.13	28.61	0.87	3.72	0.21	2.94	0.14	1.26
Sm18	Sumur	25.74	0.95	28.62	1.13	4.11	0.17	<1	-	1.11
Sd1	Sidr	29.82	1.26	32.07	1.25	3.61	0.24	<1	-	1.08
Sd2	Sidr	31.51	0.95	34.83	0.96	<2	-	2.64	0.11	1.11
Sd3	Sidr	28.74	1.16	32.42	1.16	2.94	0.25	3.65	0.22	1.13
Sd4	Sidr	33.53	1.59	31.46	1.41	4.36	0.30	3.21	0.24	0.94
Sd5	Sidr	27.44	0.92	31.98	0.83	2.16	0.16	2.09	0.17	1.17
Sd6	Sidr	30.57	0.87	36.93	1.16	<2	-	<1	-	1.21
Sd7	Sidr	29.71	1.26	33.74	0.87	<2	-	4.37	0.31	1.14
Sd8	Sidr	28.64	0.83	34.68	0.91	2.97	0.22	2.62	0.26	1.21
Sd9	Sidr	29.98	1.41	35.95	1.39	<2	-	<1	-	1.20
Sd10	Sidr	27.36	0.95	29.92	1.15	5.22	0.26	3.57	0.22	1.09
Sd11	Sidr	19.66	0.68	26.62	0.59	4.18	0.24	<1	-	1.35
Sd12	Sidr	25.51	0.96	28.44	0.89	<2	-	<1	-	1.11

Table 4. Continued.

Sample code	Sample type	Glucose (%)		Fructose (%)		Sucrose (%)		Maltose (%)		F/G
		Average	SD	Average	SD	Average	SD	Average	SD	
C1	Commercial	29.71	0.85	32.47	1.26	3.87	0.21	3.26	0.17	1.09
C2	Commercial	24.72	1.06	28.66	0.94	2.95	0.26	<1	-	1.16
C3	Commercial	26.64	1.48	27.72	0.84	<2	-	2.94	0.26	1.03
C4	Commercial	30.06	1.38	29.35	1.26	4.37	0.32	<1	-	0.98
C5	Commercial	25.67	0.95	26.35	0.90	8.63	0.57	<1	-	1.03
C6	Commercial	28.65	1.10	33.47	1.47	<2	-	<1	-	1.17
R1	Al Zahrat Al Arbaa	26.81	0.86	28.84	0.76	2.57	0.43	2.65	0.16	1.08
R2	Zahrat Al rub'a al Khali	31.13	0.93	28.65	1.13	4.25	0.18	<1	-	0.92
R3	Zuhoor Rub' al Khali	22.40	1.02	22.16	0.81	3.02	0.29	<1	-	0.99
R4	Aitman	15.07	1.42	12.34	1.08	<2	-	<1	-	0.82
R6	Arabic Gum	23.68	0.96	27.64	1.05	2.92	0.27	<1	-	1.17
R7	Talah	23.36	1.26	25.94	0.62	3.57	0.31	35.44	0.08	1.11
R8	Arabic Luban	25.52	1.07	28.65	1.12	<2	-	<1	-	1.12
R13	Qasam	25.83	1.09	29.37	0.74	<2	-	20.68	0.13	1.14

4. Conclusion

Eight rare varieties of native Omani honey, along with 18 Sumur, 12 Sidr and 6 commercial honey samples, were analyzed for their total antioxidant content and sugar profiles using a simple, rapid, potable, and disposable paper-based colorimetric device. Additionally, the pH, free acidity, and conductivity of the samples were measured by common methods. Seven rare samples showed normal sugar content ranging from 48.58% to 65.02%, with fructose (22.16%-29.37%) and glucose (22.40%-26.81%) as the major carbohydrates, which were comparable with previous reports. Only one sample, namely Aitman, showed a relatively low percentage of sugar (30.41%). Furthermore, three out of eight rare samples (Zahrat Al rub'a al Khali, Zuhoor Rub' al Khali, and Aitman) appeared to have a fructose-to-glucose ratio of less than 1, indicating their early crystallization. The total antioxidant contents of the rare samples analyzed mostly ranged from 247.15-325.41 meqGA/kg, significantly higher than those of Sidr, Sumur, and commercial samples (ranging between 100 to 200 meqGA/kg). The highest concentrations of antioxidants (>300 meqGA/kg) were found in "Al Zahrat Al Arbaa" and "Qasam" honey samples, which are expensive varieties in Oman. The acidity of rare samples ranged from 7.87 to 86.67 meq(GA)/kg); which fits the standard values set by various standardization organizations, except for Talah, which exceeded the limit of 50 meq(GA)/kg. This result aligns with previous reports and may be due to the higher organic acid content of floral origins. Sumur samples also had a free acidity higher than 50 meq(GA)/kg, while Sidr samples showed a very low acidity in the range of 5.98-25.53 meq(GA)/kg. Regarding conductivity, most varieties of Omani honey, especially Sumur, showed high conductivity values (>0.8 mS). This study provides valuable insights into the quality and characteristics of rare Omani honey, highlighting its potential for enhancing the domestic and international standing of Omani honey.

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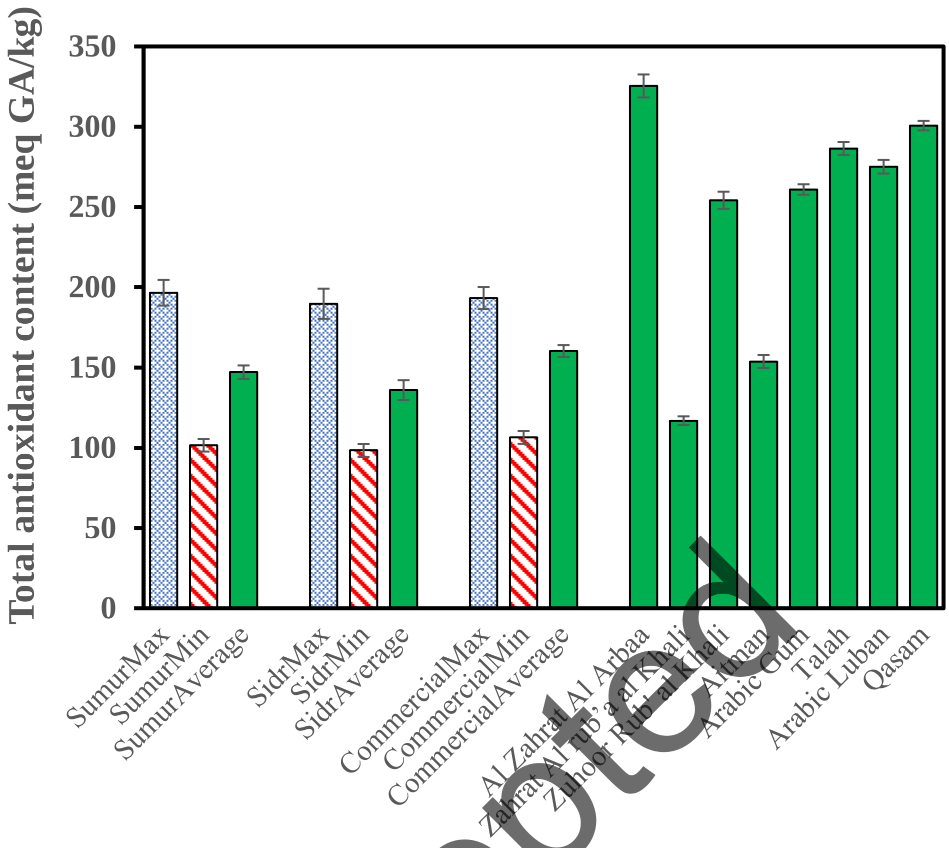
Figure Captions:

Fig. 1. Comparing the total antioxidant contents of rare honey samples with the maximum, minimum and average of obtained data for sumur, sidr, and commercial samples.

Fig. 2. Calibration graphs for the colorimetric determination of glucose using the designed PAD (optimized condition).

Fig. 3. Comparing the glucose and fructose contents of rare honey samples with the maximum, minimum and average of obtained data for sumur, sidr, and commercial samples.



Total antioxidant content (meq GA/kg)
350
300
250
200
150
100
50
0
SumurMax
SumurMin
SumurAverage
SidrMax
SidrMin
SidrAverage
CommercialMax
CommercialMin
CommercialAverage
Al Zahrat Al Arbaa
Zahrat Al rub'a al Khali
Zuhoor Rub al Khali
Aitman
Arabic Gum
Talah
Arabic Luban
Qasam

Figure 1.

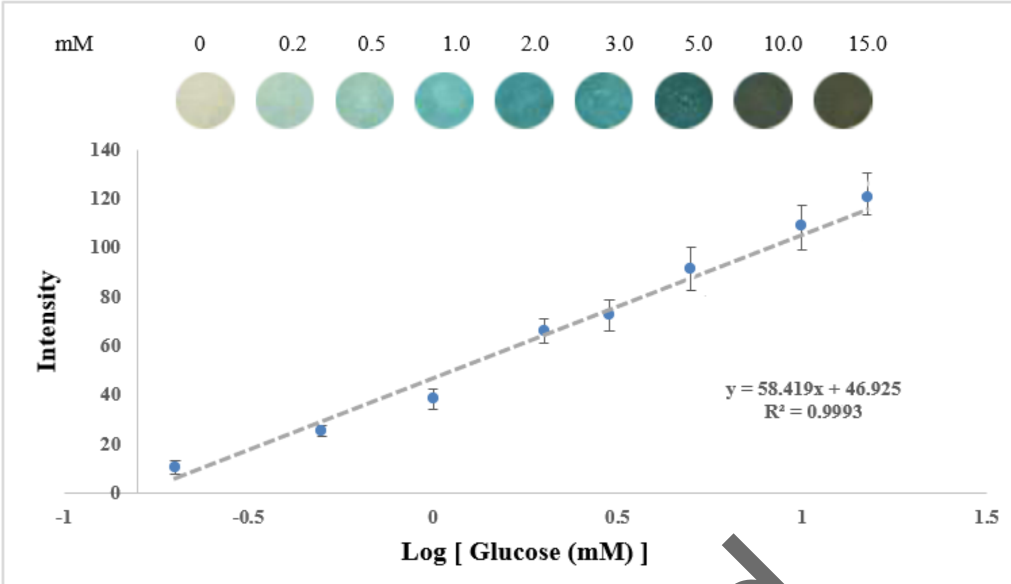
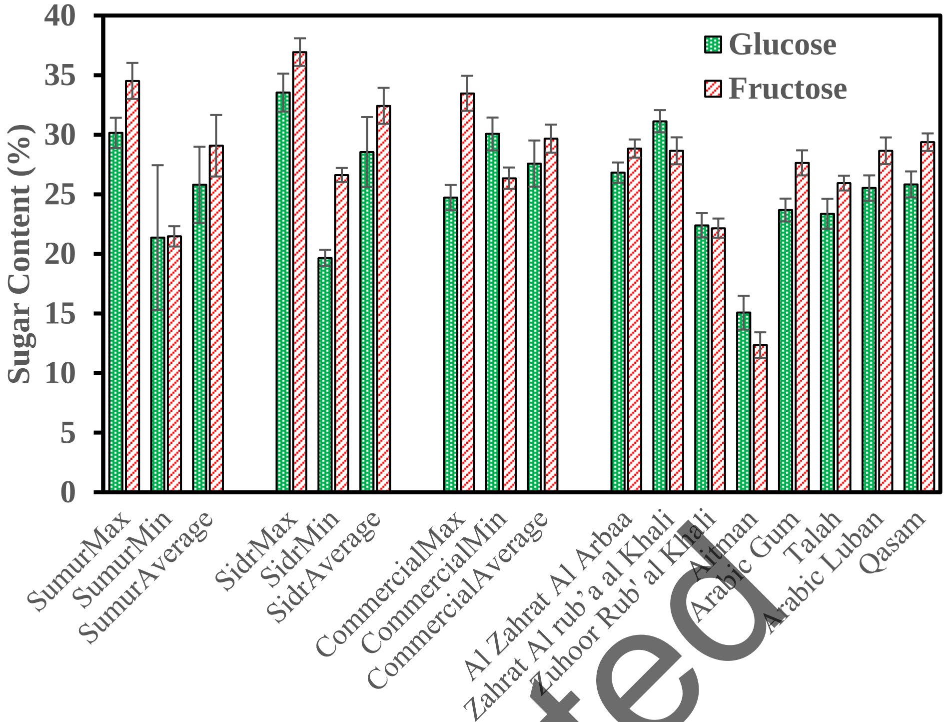

mM
0
0.2
0.5
1.0
2.0
3.0
5.0
10.0
15.0
140
120
100
80
60
40
20
0
Intensity
y = 58.419x + 46.925
R² = 0.9993
-1
-0.5
0
0.5
1
1.5
Log [Glucose (mM)]

Figure 2.



Glucose
Fructose
Sugar Content (%)
0
5
10
15
20
25
30
35
40
SumurMax
SumurMin
SumurAverage
SidrMax
SidrMin
SidrAverage
CommercialMax
CommercialMin
CommercialAverage
Al Zahrat Al Arbaa
Zahrat Al rub'a al Khali
Zuhoor Rub' al Khali
Ajman
Arabic Gum
Talah
Arabic Luban
Qasam

Figure 3.